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Remediation Strategies To Reduce Heavy Metal Uptake In Lettuce Grown In Contaminated Urban Soil

Ryley Thomas
University of Nebraska-Lincoln

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REMEDIATION STRATEGIES TO REDUCE HEAVY METAL UPTAKE IN LETTUCE GROWN IN CONTAMINATED URBAN SOIL

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by
Ryley Thomas, BS,
Environmental Studies
College of Agricultural Studies and Natural Resources

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Faculty Mentors:
Michael Kaiser, Ph.D., Agronomy
Jennifer Cooper, Ph.D., Agronomy

ABSTRACT

Urban soils are increasingly used to produce food for local consumption, which requires at the same time management strategies that prevent the plant uptake of potentially present contaminants. This study was conducted to test different soil amendments for their ability to retain lead (Pb) and arsenic (As) within the soil matrix. The analyzed soil was taken from a potential community garden lot near a railroad in Lincoln, NE where elevated concentrations in Pb and As had been detected. Ponderosa pine biochar and spent coffee grounds were used as soil additives because of their documented chemical reactivity towards soil cations or anions. In a greenhouse experiment, romaine lettuce (*Lactuce sativa*) was grown in the soil collected from the community garden location with or without addition of biochar or coffee grounds. Lettuce was chosen due to its short growing season and high relevance for urban food production. The biochar amendment resulted in the lowest concentrations of both Pb and As in the leaves and stems parts of the plant compared to the garden control and the coffee ground amendment. In contrast, the coffee grounds had the highest concentrations of Pb and As throughout all three tested parts of the plant (leaf, stem, and roots) across treatments. These findings suggest that biochar application to urban soil presents a potential successful strategy to prevent the plant uptake of contaminants such as As and Pb. In a next step, this strategy needs to be tested for its usefulness towards a large variety of plants grown for food production in the urban environment.

KEYWORDS: urban soil, remediation, community garden, lead, arsenic, coffee grounds, biochar, lettuce

APPRECIATION

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INTRODUCTION

Within the city of Lincoln, NE and other urban environments, many food deserts exist. According to the US Department of Agriculture (USDA) a food desert is a low-access community of at least 500 people or a community with at least 33% of the population living more than one mile from a supermarket (USDA, 2019). As of 2016, an estimated 325,000 Nebraskans live in food deserts (Senator Hansen, 2016), which accounts for about 17% of the states' population. One way to address these food deserts is urban food production, which would help to provide locally produced food to growing populations in and around urban centers. Food produced from community gardens on urban land is one method to compensate for food deserts and can help to reduce the effects of obesity and malnutrition by providing a low-cost option for healthy produce. According to the executive director of Community Crops, David Garbarewitz, across Lincoln hundreds of acres of under-utilized areas exist that could be turned into community gardens (D. Garbarewitz, personal communication, July 1, 2019).

However, urban areas can be contaminated with Pb, As, and other heavy metals. For example, according to the Nebraska Department of Health and Human Services (2013), nine out of twenty residential yards in the North Bottoms neighborhood tested, contained over 400 mg/kg Pb^{2+}. The North Bottoms neighborhood is located directly southwest of the intersection of Salt Creek Roadway and North Antelope Valley Parkway. This neighborhood is considered a food desert. The Nebraska Department of Environmental Quality (2013) recommends levels of less than 100 mg/kg for Pb^{2+} and 10.2 mg/kg for As in garden soils. Consequently, high Pb and As concentrations can be expected in crops, fruits or vegetables if grown in contaminated urban soil. Pb and As can negatively impact human health. According to the Agency for Toxic Substances and Disease Registry (2013), children are particularly sensitive to Pb poisoning. Pb targets the nervous system and can cause attention deficit hyperactivity disorder, lower intelligence quotient, hypertension, renal effects, and reproductive problems (ToxGuide, 2007). The effects of Pb can be transferred from generation to generation, affecting a community for years. As of 2016, worldwide exposure to Pb has accounted for 540,000 deaths and 63.8% of the global burden of idiopathic developmental intellectual disability (WHO, 2016). As causes the same effects as Pb in both children and adults and is a carcinogenic. The organic form of As is considered less harmful than the inorganic form (ATSDR, 2015). When inorganic As is inhaled it may cause respiratory irritation, nausea, skin effects, and increased risk of lung cancer (ToxGuide, 2007). When ingested orally, inorganic As may cause nausea, vomiting, diarrhea, cardiovascular effects and encephalopathy (ToxGuide, 2007). The long-term effects of oral exposure are hyperpigmentation and hyperkeratosis, corns, warts, peripheral neuropathy and may increase risk of skin cancer, bladder cancer, and lung cancer (ToxGuide, 2007).

Pb and As can accumulate in soil due to atmospheric deposition. The USDA defines atmospheric deposition as the process whereby precipitation (rain, snow, and fog), particles, aerosols, and gases move from the atmosphere to the earth's surface. This type of contamination is common in industrialized areas including freeways and railroads. The impact of vehicular traffic on soil quality was outlined in a study by França et al. (2016), who found that soil had concentrations of Pb^{2+} up to 160 mg/kg in high traffic area. Heavy metals can also enter the soil from the parent material and fertilizers. There are two human exposure pathways for heavy metals, soil to person and soil to plant to person (EPA, 2011). This study will focus on decreasing the uptake of Pb and As from soil to plant.

Pb^{2+} typically has very low solubility in soil (Peryea, 2001). Pb^{2+} is the most common form of

Pb found in soil (Xintaras, 1992). In soil with pH >7.0, Pb^{2+} is not highly soluble (Peryea, 2001). Pb^{2+} typically has very low mobility in soil, however, in highly contaminated soil, the uptake of this heavy metal by the plant roots can exceed levels tolerated by most plants resulting in stress symptoms including death (Peryea, 2001). The actual mechanism by which Pb enters the root is still largely unknown (Porrut et al., 2011), but the uptake of Pb occurs via the apoplastic pathway or via calcium (Ca^{2+}) permeable channels through the roots. High Pb^{2+} concentrations have been found near the apical area; the apical area is found at the very tip of the root and is the youngest part of the plant. The young root cells in the apical area have very thin cell walls; this in conjunction with the lower rhizodermic pH facilitates Pb uptake by the plant as lower pH increases the solubility of the Pb (Porrut et al., 2011). Pb commonly stays in the roots due to the Casparian strip blocking it (Porrut et al., 2011). The Casparian Strip is an apoplastic diffusion barrier that forces selective transport across the cell's endodermis (Li et al., 2018) and is a physical barrier (apoplast) that regulates water and soluble metals or nutrients before they enter the xylem (Porrut et al., 2011).

Pb^{2+} can be found in soil in four different forms: i) free metal cation, complexed by inorganic constitutes (some common examples are: PbS, $PbSO_4$,$PbCO_3$, Pb_3O_4 (IARC, 2006)), ii) organometal complex (chelate), iii) absorbed to surfaces of soil minerals as a positively charged ion, or iv) as part of the soil parent material (Porrut et al., 2011, Peryea 2001). Predominate insoluble Pb^{2+} compounds are lead phosphates and lead carbonates, they form when pH is above 6 (Wuana & Okieimen, 2011). Lead sulfates are the most stable compounds but require reducing conditions and high levels of sulfides (Wuana & Okieimen, 2011). Common chelators that bind to Pb^{2+} are different types of organic acids (Porrut et al., 2011). Pb can also bind to Fe-oxides, organic matter, or clay minerals (Porrut et al., 2011). Since Pb^{2+} has a strong ability to bind with organic and inorganic colloids it can be available for plant uptake causing severe plant damage (Porrut et al., 2011). Colloids are defined as, "solid particles with an equivalent spherical diameter between 1 and 1000 nm dispersed in a liquid phase" (IUPAC, 1997). These colloids' small size makes them susceptible to plant uptake (Kretzschmar & Daniel, 2005). Pb^{2+} was found to bind with iron rich and sometimes aluminum rich colloids and the organic matter associated with these particles (Löv et al., 2018). However, the molecular speciation of bound Pb^{2+} to particulates and colloids in the water phase is largely unknown. (Löv et al., 2018). Plant uptake can be impacted by soil pH, soil particle size, CEC, root surface area, and root exudation (Porrut et al., 2011).

As is toxic to plants and enter the food chain by mobilization in water. Once mobilized in water, As can transfer between phases. (Moreno-Jiménez et al., 2012). As is most mobile in soil with a pH under 3 or above 7. (Moreno-Jiménez et al., 2012) As is more soluble and mobile in soil than Pb. As becomes increasing mobile with moisture (Peryea, 2001). The two types of As found in soil are arsenate (As (V), AsO_4^{3-}) and arsenite (As (III), predominately AsO_3^{3-}). As(V) is the form of As that is commonly found in aerobic, oxidizing conditions and changes to As(III) under reduced conditions. Iron oxides and hydroxides have a high affinity for both As(III) and As(V). As(V) has also been found to form week bonds with Ca^{2+}, Al^{3+}, and Fe^{3+} and then, adsorb onto the particulate surface, reaching maximum adsorption at pH 5 (Fleming et al., 2013, Cao & Ma, 2004).

Plants can take up As(V) in small amounts. Once in the plant, As(V) is reduced to As(III). The amount of phosphorous (P) influences the uptake of As(V) and determines how toxic it will be to the plant (Moreno-Jiménez et al., 2012). Arsenate (As(V): AsO_4^{3-}) is an analog for phosphate

(PO_4^{3-}), therefore, it can be up taken by the phosphate transport system in plants (Esteban, Carpena, Meharg, 2003). Plants deal with As contamination by complexation and compartmentalization of the toxin. During complexation, the plant produces phytochelatin which complexes inorganic As, deactivating its toxic effects (Moreno-Jiménez et al., 2012). Compartmentalization is the storing of this complexed As in the vacuoles of the root cells (Moreno-Jiménez et al., 2012). This process can result in symptoms of phytotoxicity such as growth inhibition, chlorophyll degradation, nutrient depletion and oxidative stress (Moreno-Jiménez et al., 2012).

For soils contaminated with heavy metals, the main remediation strategies include containment, extraction, and immobilization (Liu et al., 2018). Bioremediation is the use of microbes and other living organisms to immobilize heavy metals in the soil, while phytoremediation uses plants to uptake the heavy metals, followed by removal of the plants and heavy metals from that area. Bioremediation and phytoremediation techniques can work in conjunction. In areas with heavy As and Pb contamination, it is a common practice to scrape off the top few inches and discard that soil, then use phytoremediation to capture the remaining contaminant. Since soil is continually exposed to As and Pb atmospheric deposition, the remediated soil will have to be continually monitored, in places of high heavy metal atmospheric pollution (EPA, 2000). Soil amendments are considered bioremediation and are a good option for remediation because there is little work or cost after the additions are completed (Oliveira et al., 2017). Soil amendments can be defined as “any material added to soil to improve its physical properties” (Davis & Whiting, 2013). Soil amendments can be organic or inorganic. Organic soil amendments are associated with beneficial effects such as high plant available water holding capacity, higher cation-exchange capacity, lower bulk density, and promotion of beneficial microorganisms (Bulluck et al., 2002). The two amendments that are used in this experiment are organic biochar and spent coffee grounds.

As defined by Tang et al. (2013) biochar is biomass that has been decomposed under pyrolysis or high heat under oxygen-limited conditions and is mainly influenced by the type of biomass and the pyrolysis. Biochar amendments work in the soil by creating a more negative charge on the soil surface. This is partly due to increasing the cation exchange capacity (CEC) and decreasing zeta potential. Zeta potential is the electro kinetic potential in colloidal dispersions (Tang et al., 2013). A colloidal dispersion occurs anytime particles in two or more phases (liquid, gas, solid) interact. Zeta potential is a measurement of the repulsion or attraction between the molecules within that dispersion (University of Colorado, 2015). Zeta potential is impacted by pH. Zeta potential is lower in higher pH and higher in a low pH. As is most mobile at a higher pH while Pb^{2+} is most mobile at a lower pH. Zeta potential that is higher results in stabilization of the solution while zeta potential that is lower results in flocculation (University of Colorado, 2015). As will be less likely to flocculate due to zeta potentials while Pb^{2+} is more likely to flocculate due to zeta potentials. These flocs of Pb^{2+} will be more difficult to change forms and dissolve into the liquid stage (Khoshnevisan & Barkhi, 2015).

Biochar's surface has functional groups that are available for heavy metal complexation (Tang et al., 2013, Lu et al., 2012). Biochar releases phosphate and carbonate ions, which are acting alkaline. Zhang et al. (2016) found that biochar amended soils were able to increase available phosphate, which were found to be effective at immobilizing Pb^{2+} (Mignardi et al., 2012). Biochar increases soil pH and thus decreases the mobilization of heavy metals (Tang et al., 2013). Due to the strong buffering capacity of phosphate and carbonate ions, the soil pH remains unchanged, even with a high concentration of Pb^{2+} present, as Pb^{2+} complexes the phosphate and carbonate ions (Inyang et al., 2012). Furthermore, Inyang et al. (2012) found that almost all Pb^{2+} was absorbed by biochar when the Pb^{2+} equilibrium concentrations were fairly low in wastewater. There was 64% to 92% removal of Pb^{2+} from wastewater when filtered through biochar made from wood (Inyang et al., 2012). The efficiency of Pb^{2+} removal was due to increased soil pH and Pb^{2+} precipitation in the form of oxides, carbonates, phosphates, and hydroxides (Oliveira et al., 2017). Biochar sourced from wood usually has a higher surface area than from other feedstocks, which may result in a higher holding capacity for Pb^{2+} ions. A study by Mohan et al. (2007) also showed that As was 70% removed from an aqueous solutions with an application of 1% of biochar from oak bark (Oliveira et al., 2017). The mechanism by which As is adsorbed by biochar is complexation with released cations and electrostatic interaction with the functional groups (if positively charged) (Agrafioti et al., 2014, Li et al., 2017).

An application of biochar has been found to sequester carbon in soil, improve overall soil health, reduce nitrogen loss, and increase in yields (Verheijen, Jeffrey, Bastos, van der Velde, Diafas, 2010, Steiner et al., 2014). Biochar has promising characteristics, is non-toxic, and easy

to apply, however, it is less cost effective and often less easily accessible.

In contrast to biochar, spent coffee grounds are a cost-effective and plentiful waste product, however, research into its effectiveness at remediating metal contaminated soils is sporadic. According to Esquivel & Jiménez (2011), coffee grounds are 50% of the waste from coffee production and can contain P^{-3}, Ca^{2+}, Al^{3+}, and Fe^{3+} ions, which might increase As immobilization and reduce the plant uptake of As (Moreno-Jiminez et al., 2012, Mussatto et al., 2011). A study by Bravo et al. (2012) found coffee grounds to be a source of hydrophilic bioactive compounds (antioxidants). Thus, the antioxidants in the coffee grounds may attract Pb^{2+}. A study by Kim et al. (2014) found that when coffee grounds and coffee ground biochar was added to a soil that had been treated with acid mine drainage water, 99% of Pb^{2+} was removed. The high removal of Pb^{2+} was assumed to be due to Pb^{2+}'s higher electronegativity constant (2.3) than the other heavy metals (Kim et al., 2014). Coffee grounds have a high surface area (300-1000 m^2/g) and have shown metal-chelating activity (Kovalcik et al., 2018). Also, coffee grounds have been found to be sources of carboxyl and hydroxyl groups that have been shown to help stabilize Pb^{2+} (Kovalcik et al., 2018, Uchimiya, et al., 2012). Coffee grounds are also very high in potassium (K^+) (1225 mg/kg K). A study by Porrutt et al. (2011) stated that Pb^{2+} may enter plants through the same channel as K^+ due to their similar radii. The use of coffee grounds as a soil amendment or remediation material warrants further research and may shed light on potentially negative effects of caffeine and organic acids on plant growth. Another unknown for the coffee grounds is how the addition to the soil will affect moisture retention, which may impact the speciation of As.

The overarching research question in this study is, will amending soil with biochar or spent coffee grounds immobilize Pb^{2+} and As in the soil, and limit their uptake into plant tissues? To clarify this, I will analyze contaminant immobilization in a contaminated potential garden soil, in comparison to the same soil but with amendments and a control soil, while growing lettuce. By planting lettuce in contaminated garden soil, I will be able to quantify the amount of Pb^{2+} and As that is retained in the soil, in addition to measuring uptake in different parts of the plant (i.e. leaves, stem, roots). If the amendments are successful, the Pb and As levels will be below recommended human consumption levels by the Food and Drug Administration and the United States Institute of Medicine Panel on Micronutrients in the lettuce but remain high in the contaminated garden soil. The contaminated garden soil was sourced from a site near railroad tracks with elevated levels of Pb and As levels. Our uncontaminated control soil came from a long-term corn only rotation plot. I used a greenhouse experiment analyzing the following treatments: i) Pb and As free agricultural soil as control, ii) Pb and As contaminated garden soil, iii) Pb and As contaminated garden soil mixed with biochar, and iv) Pb and As contaminated garden soil mixed with spent coffee ground. Harvested plants were separated into leaves, roots, and stem. The soil was analyzed as bulk soil and root bound soil. Bulk and root soil, and plant leaves, roots, and stem were analyzed for Pb and As concentrations post experiment in addition to imaging the distribution of Pb and As with use of a scanning electron microscope with energy dispersive x-ray (SEM-EDX) spectroscopy for

mapping of the elements, and their associations.

I hypothesized lower plant uptake of Pb and As in treatments with soil amendments (biochar and coffee grounds), however, I expected biochar to be more effective than coffee ground because due to higher sorption capacities. I also hypothesized that the highest levels of As and Pb are found in the roots followed by the stem because of closer contact with the soil and higher concentrations of heavy metals along the petiole and veins. I expected the least contamination in the blade tissue because of the physiological uptake barriers within the plant minimizing heavy metal concentration in leaves.

MATERIALS AND METHODS

Soil sampling and amendments

The soil collection site is a potential community garden in Lincoln, Nebraska near railroad tracks at 5th and E Street, and was found to have high levels of Pb and As by the Lancaster County Health Department (Table 1). The Lancaster County Health Department reported Pb levels ranging from 63.3 mg/kg to 506 mg/kg. Only the Southeastern quadrant of the North parcel (506 mg/kg) was tested above the Environmental Protection Agency (EPA) recommended

Pb levels, which should be <400 mg/kg. This a recommendation for areas where children may reside. The USGS found the average level of soil Pb in the state of Nebraska is 18.9 mg/kg with a sample size of 130 samples. The Lancaster County Health Department test showed As levels ranging from 12.1 mg/kg to 75.7 mg/kg. The EPA standard is that 99% of soil tested should be under 13.7 mg/kg of As to be considered uncontaminated. Only the test area with the lowest levels was below this 13.7 mg/kg recommendation.

Based on this survey we collected soil from the Northeast corner of the lot, where the highest contamination was reported. We collected four independent field composites from the top 12 in. of soil using a shovel and buckets (avoiding grass and roots), sampling approximately every 5 ft. The soil was stored at room temperature in buckets until further processing.

Spent coffee grounds were obtained from the Mill Coffee in Lincoln, Nebraska and represents a random subsample of daily by-products from commercial coffee brewing. Prior to soil addition, coffee grounds were dried at 60˚C for two days, paper filters were removed, and aggregates were gently broken up. For our biochar addition, we selected high temperature ponderosa pine (*Pinus ponderosa*) biochar (15 min. at O_2 limited 760ºC, 15 min cooling, quench with water for final cooling and dust prevention) produced at High Plains Biochar (Laramie, WY). Our control soil was collected in the same manner as the garden soil, between rows of a corn field at the South-Central Agricultural Laboratory in Clay Center, Nebraska.

Table 1. *Survey by Lancaster County Health Department of 5th and E community garden site, showing heightened levels of Pb and As. Please note that the site-specific background value is 10.2 mg/kg, and lead levels for gardening are recommended to be less than 100 mg/kg. ND=not detected, NG = no goal.*

Analyte	Units	VCP Remediation Goals Residential Soil	North				South			
			NE	NW	SE	SW	SW	NW	SE	SW
Arsenic	mg/kg	0.39	73.4	73.4	23.7	32.2	19.6	75.7	12.1	23.6

Barium	mg/kg	3,800	249	249	215	399	202	299	202	198
Cadmium	mg/kg	18	1.2	1.2	1.0	1.3	1.3	3.0	0.9	3.6
Chromium	mg/kg	NG	14.6	14.6	13.2	9.2	11.6	14.5	16.2	13.2
Lead	mg/kg	400	168	168	231	506	100	177	78.3	63.3
Selenium	mg/kg	98	ND	ND	ND	ND	1.5	ND	ND	ND
Silver	mg/kg	98	ND	ND	ND	ND	ND	ND	ND	ND
Mercury	mg/kg	5.9	0.11	0.11	0.10	0.19	0.13	0.88	0.07	0.2

Greenhouse Experiment

Treatments were established in a greenhouse experiment on April 10, 2019, in a randomized complete block design. The treatments were: i) Pb and As free agricultural soil as control, ii) Pb and As contaminated garden soil, iii) Pb and As contaminated garden soil mixed with biochar, and iv) Pb and As contaminated garden soil mixed with spent coffee ground. Each treatment was replicated four times, for a total of 16 pots. Visible plant material was removed from the control soil and each of the four soil field replicates was hand mixed to ensure homogeneity. Large pieces of visible coal (approximately larger than 5 mm), likely deposited from nearby the train tracks, were removed from the garden soil, along with visible plant material, before homogenization of the four field replicates by hand. Per manufacturer estimates, biochar was expected to contain 85% carbon, and added at a rate of 40 t biochar/ha (47 g biochar/pot, and 42 g carbon/pot). Because we are evaluating the effectiveness of carbon to remediate heavy metals, we decided to add equivalent amounts of carbon (~42 g carbon/pot) from each soil amendment, based on the 40 t/ha biochar addition rate, rather than equivalent weights. Coffee grounds were estimated to contain 60% carbon (Pujol et al., 2013).

Coffee grounds were then added to each pot at a rate of 57 t/ha (66.6 g coffee/pot, 42 g carbon/pot). Amendments were hand mixed with each field replicate of the garden soil prior to ensure homogeneity. Each treatment was lightly packing into a black plastic square pot (11.4 cm x 11.4 cm x 11.4 cm; V = 1493 cm^3), placed into a tray filled with deionized water, and allowed to become saturated through capillary action. The soil was kept wet for one week to overcome the initial hydrophobicity of the coffee and biochar and was drained thereafter

After two days of draining, soil moisture was determined and pots were watered to 55% of saturated water holding capacity, followed by planting of Johnny's Romaine Lettuce (*Lactuce sativa*) Green Forest MT0 0G-Pellet seeds. Three seeds were planted in each pot to a depth of approximately 1/8 in. and thinned to one plant/pot after germination and establishment, on April 28, 2019. Extra plants were germinated using pasteurized greenhouse soil for back up plants in case of unsuccessful germination or delayed growth. Two of the control replications did not have any plants on April 28, 2019 and received a transplant from the greenhouse soil propagated backup plants. The transplanted plants were less than 60 grams. On May 22, 2019 approximately 6 weeks after starting the experiments, the additional germinated plants were added to all pots of the soil mixed with spent coffee ground because the coffee plants were stunted in size with leaves less than 1 cm. Each transplant was about 60 grams.

Plants were watered every other day to maintain all treatments at 55% water holding capacity with deionized water. Soil moisture was determined by weighting each individual pot, and comparing to the known dry weight values, until plants were large enough to start to interfere with the weights (at approximately 1.5-inch leaves). After that time, a set of controls for each treatment – that contained no plant – were maintained in the greenhouse under the same conditions as the experimental pots and used to estimate water losses from each treatment. Due to insect infestation, Pylon and Azatin pesticides were applied on May 24, 2019. Bug tape was added on May 30, 2019 in each pot using plastic stakes.

Plant and Soil Preparation

The experiment was terminated on June 11, 2019, after all plants had leaves that reached 3 inches when measured from stem to tip. Aboveground biomass was removed by cutting the plant at the soil level. Leaves were removed from the stem and separated. Intact roots were gently removed from the soil (most roots were within two inches of the stem), with any soil that clung to the removed roots considered as "root soil". Root soil was washed from the roots with deionized water into a glass jar, dried in the oven at 105 ˚C for 48 hours. The remaining soil in the pot was considered as bulk soil, weighted, homogenized and a portion dried at 105 ˚C for 48 hours to determine overall dry mass. The leaves, stem, and roots were washed under cool, running tap water for thirty seconds, and placed in a deionized water bath for one minute. After one minute, the deionized water in the bath was replaced and the stems, leaves, and roots entered the bath for a second minute, were patted dry and weighed (Uzu et al., 2010). One stem, leaf, and root for each treatment were set aside and dried under vacuum for 72 hours for SEM-EDX analyses. Remaining plant tissues were oven dried at 50 ˚C for 72 hours. Following drying, plant tissues were weighted, and all non-SEM-EDX samples were ground using a mortar and pestle. The roots and stems from the replicated samples of each treatment were combined to ensure large enough sample size for digestion. For the treatments receiving coffee grounds, the leaves from the original plant replicates and from the transplanted plant replicates were combined as well. All ground samples were placed in a freezer overnight in preparation for heavy metal digestions.

Initial Characterization, Soil and Plant Analyses

Initial pre-experiment characterization of control soil, garden soil, biochar and coffee ground were conducted prior to initiating the experiment at the University of Nebraska-Lincoln Soil Physics Laboratory and Ward Laboratories in Kearney, NE. Due to the short duration of the experiment, we analyzed for plant available forms. Soil pH and soluble salts were measured using a 1:1 soil:water ratio. Organic matter content was measured by loss on ignition. Nitrate was extracted with 2M KCl and measured with a Lachat Quickchem Flow Injection Analysis System (Hach, Loveland, CO). Phosphate (PO_4^{3-}) and sulfate (SO_4-S) were extracted with a melich-3 and measured colorimetrically (phosphate) or with an iCAP RQ Inductively Coupled Plasma-Mass Spectrometer (Waltham, MA) (ICAP) for plant available content. Potassium, calcium, magnesium, and sodium were measured for plant available content using an ammonium acetate extraction and analysis on an ICAP. Zinc, iron, manganese, and copper were measured using a plant available diethylenetriaminepentaacetic acid (DTPA) extraction method and analyzed on an ICAP. Boron was extracted with hot water and analyzed on an ICAP. Total carbon and nitrogen air dry samples were ball milled on a SPEX Sample Prep 8000 D Mixer/Mill (Metuchen, NJ) and measured by dry combustion on a Thermo Scientific Flash 2000 Organic Elemental Analyzer (Waltham, MA).

After freezing overnight, soil and plant tissues underwent microwave digestion according to EPA 3052 in preparation for Pb and As analysis. Approximately 0.24 g was used for soil digestions, while approximately 1 g was needed for plant tissue samples. Briefly, hydrochloric and nitric acids were added in combination with hydrogen peroxide to soil and plant tissue samples and allowed to react for 15 minutes in microwave extraction Teflon vessels (CEM

Microwave Technology, Buckingham, UK) plugged with MARS Xpress System Teflon vessel plugs (model number12020, CEM Microwave Technology). Teflon caps were placed on top of the plugs and tightened using the MARS Xpress manual torque tool (model 185245 CEM Microwave Technology) and placed into a 40-slot turntable holder. All samples were microwaved in the MARS Xpress system (CEM microwave technology), on the TOTALS setting for 35 minutes. Samples were cooled in the fume hood, transferred to a 50 mL sample tube (Fisherbrand, Hampton, NH) and diluted deionized water to 50 mL. Following 15 minutes of settling, sample were filtered and analyzed by inductively coupled plasma mass spectrometer (ICP-MS) for Pb and As.

Scanning Electron Microscope – Energy Dispersive X-ray

Following vacuum drying, small pieces of leaf, stem or root biomass were mounted onto carbon tape and sputtered for ninety seconds with gold to prevent charging of the surface which can disrupt picture clarity. Samples were placed in the SEM-EDX (FEI Nova NanoSEM450, Thermo Fisher Waltham, MA) and images were taken of the stem, leaf, and root from the garden soil treatment. Major elements present were auto identified elements in addition to Pb and As. The resulting list of elements that were identified are: carbon, nitrogen, gold (from sputtering), oxygen, Pb, and As.

Statistical Analysis

One-way ANOVA tests for significance were run on the leaf, bulk and root soil concentrations for both Pb and As. The Holm-Sidak test was used for the separation of means on all root soil and leaf concentration analysis. While the Dunn test was used for the separation of means on the root soil concentration analysis.

RESULTS

Soils and Amendments

The garden soil and the control agricultural soil as well as the biochar and spent coffee grounds were analyzed for basic chemical properties that are presented in Table 2. As expected, the agricultural, control soil had significantly lower concentrations in Pb and As then the garden soil. The garden soil had a substantial percent of carbon (33.4%) compared to the agricultural soil (2.9%) this is a 10% increase in carbon. While the both the biochar (60.2%) and coffee (49.3%) amendments had substantial amounts of carbon, the high carbon level of the garden soil could have decreased this effect with the added carbon. The garden soil (0.84%) also had a higher percent nitrogen than the agricultural, control (0.26%) soil by about 3%. The garden soil (0.26%) had a slightly greater percent nitrogen than the biochar (0.28%) did. This could also have decreased the effectiveness of the additional nitrogen. The biochar amendment showed a substantial concentration of nitrate (2042 mg/kg) and a neutral pH (7.7 pH). The coffee amendment showed a substantial concentration of potassium (1225 mg/kg), magnesium (333 mg/kg), nitrogen addition (2%), carbon addition (49.3%), and an acidic pH (5.08pH). The calcium additions in the amendments were not substantial as both soils had high levels of calcium already. Potassium, magnesium, nitrogen, carbon and calcium are generally plant available nutrients and could have helped the lettuce to grow.

Table 2. *Properties of control soil, garden soil, biochar and coffee (n=4)*

Treatments	Control soil	Garden soil	Biochar	Coffee
Carbon (%)	2.9 (±0.03)	33.4 (±1.47)	60.2	49.3
Nitrogen (%)	0.26 (±0.01)	0.84 (±0.03)	0.28	2.00
pH	7.18 (±0.05)	6.68 (±0.02)	7.70	5.08
CEC* ($cmol_c$/kg)	13 (±0.29)	14 (±0.26)	2	7.8
Salt (mohm/cm)	0.02 (±0.03)	0.31 (±0.02)	0.47	0.68
Nitrate (mg/kg)	13.5 (±1.12)	34.8 (±3.38)	2042	0.90
Potassium (mg/kg)	244 (±14.13)	615 (±6.14)	192	1225
Calcium (mg/kg)	2101.25 (±42.88)	2135 (±46.54)	248	347
Magnesium (mg/kg)	202 (±4.29)	220 (±2.66)	26	333
Iron (mg/kg)	n/a	n/a	5.8	2.7

Phosphorus (mg/kg)	92 (±1.11)	50 (±0.25)	13	63
M-3 Sulfate ppm S (mg/kg)	n/a	n/a	3.1	44.6
Lead (µg/g)	13.5 (±0.78)	94.8 (±3.54)	n/a	n/a
Arsenic (µg/g)	4.4 (±0.17)	39 (±1.23)	n/a	n/a

*CEC = cation exchange capacity

<u>Mass distribution of soil and plant biomass</u>

Figure 1 looks at both the mass distribution of the soil (Fig 1A) and the plants (Fig 1B). In all treatments, the root soil (0.3 – 2.6%) was substantially smaller than the bulk soil (97.4-99.7%). The garden + biochar amendment had the highest root soil mass (2.6%) and the garden + coffee had the smallest (0.3%), this is almost a 9% difference. In all treatments, the leaves (64-74%) made up the largest portion of the total plant biomass, followed by the roots (18-21%), then the stem (7-16%). Control and garden +biochar had largest leaf biomass, while stems were smaller in these two treatments. The garden and garden + coffee treatments had the largest stems. Garden + biochar had the largest proportion of roots over the other treatments.

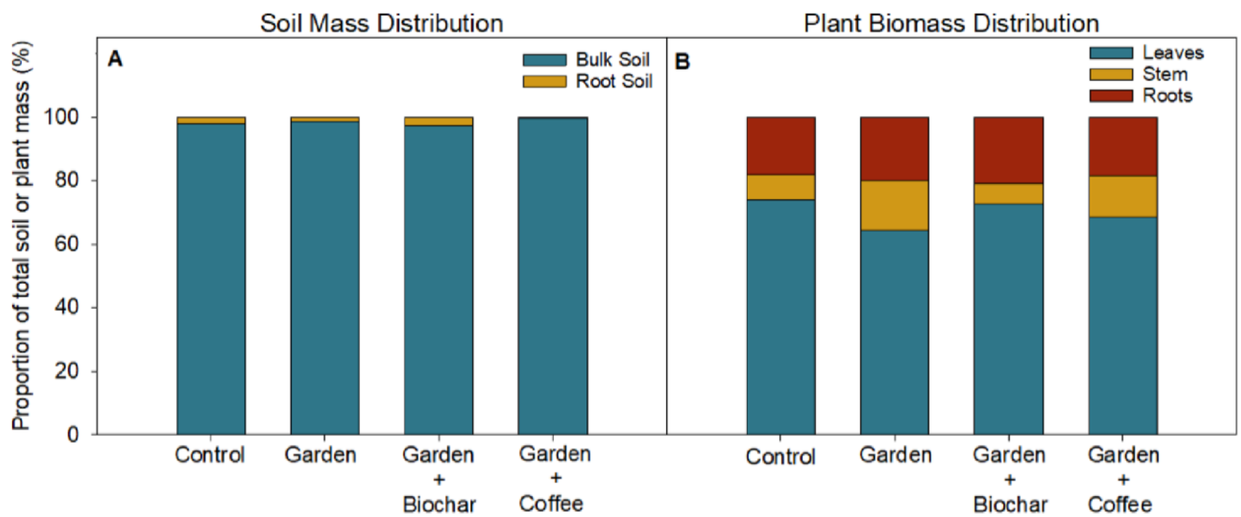

***Figure 1.** Mass distribution of bulk and root soil within total soil mass (A), and distribution of leaves, stem, and roots within total plant biomass (B).*

Soil

As expected, the control had the lowest concentration of both Pb and As in both the bulk soil and root soil. (Fig. 2A & 2B) It is difficult to find statistical difference in soil due to its high variability. However, there were some similarities. The garden + coffee and garden + biochar were more similar due to the majority of the Pb and As remaining in the bulk soil. (Fig. 2A & 2B) The garden and garden + biochar treatment were more different due to the median concentrations of Pb and As in the root soil. (Fig 2A)

Lead and Soil

For the garden and garden + biochar treatments, the concentration of mean Pb in bulk soil (39-41 µg/g) was higher than the root soil concentrations (61-64 µg/g), indicating accumulation of Pb in the root zone (Fig. 2A). The control (bulk soil: 12.8 µg/g, root soil 10.4 µg/g) and garden + coffee (bulk soil: 48.0 µg/g, root soil: 45.9 µg/g) had similar concentrations of Pb between bulk and root soil regions, indicating no or limited mobilization of Pb to, or plant uptake of Pb once in the root zone. However, statistically, there was no difference between the bulk and root soil for the garden + biochar and garden + coffee treatments. Garden soil Pb concentrations were significantly higher in the root soil than the bulk soil. Conversely, control agricultural soil had significantly higher Pb in the bulk soil than the root soil (Fig. 2A).

Arsenic and Soils

The control soil had significantly higher concentrations of As in bulk soil (3.8-4.5 µg/g) than root soil (3.1- 4.1 µg/g). (Fig. 2B) The control as expected had an overall lower concentration of As than the garden soil. The garden root soil (20.1 -40.4 µg/g) showed significantly higher As concentration than the bulk soil (12.2-17.7 µg/g), this is similar to how the Pb acted. (Fig. 2A & 2B) This would indicate accumulation in the root zone (Fig. 2B). For neither the garden + biochar or garden + coffee was there a significant difference in the concentrations of As between the bulk and root soil. The garden + biochar bulk soil (11.6-22.9 µg/g) concentrations of As were similar to the root soil (15.2-37.6 µg/g) concentrations. (Fig. 2B) The garden + coffee fairly similar concentrations of As between the bulk (18.8-20.2 µg/g) and root soil (11.3- 25.7 µg/g). (Fig. 2B)

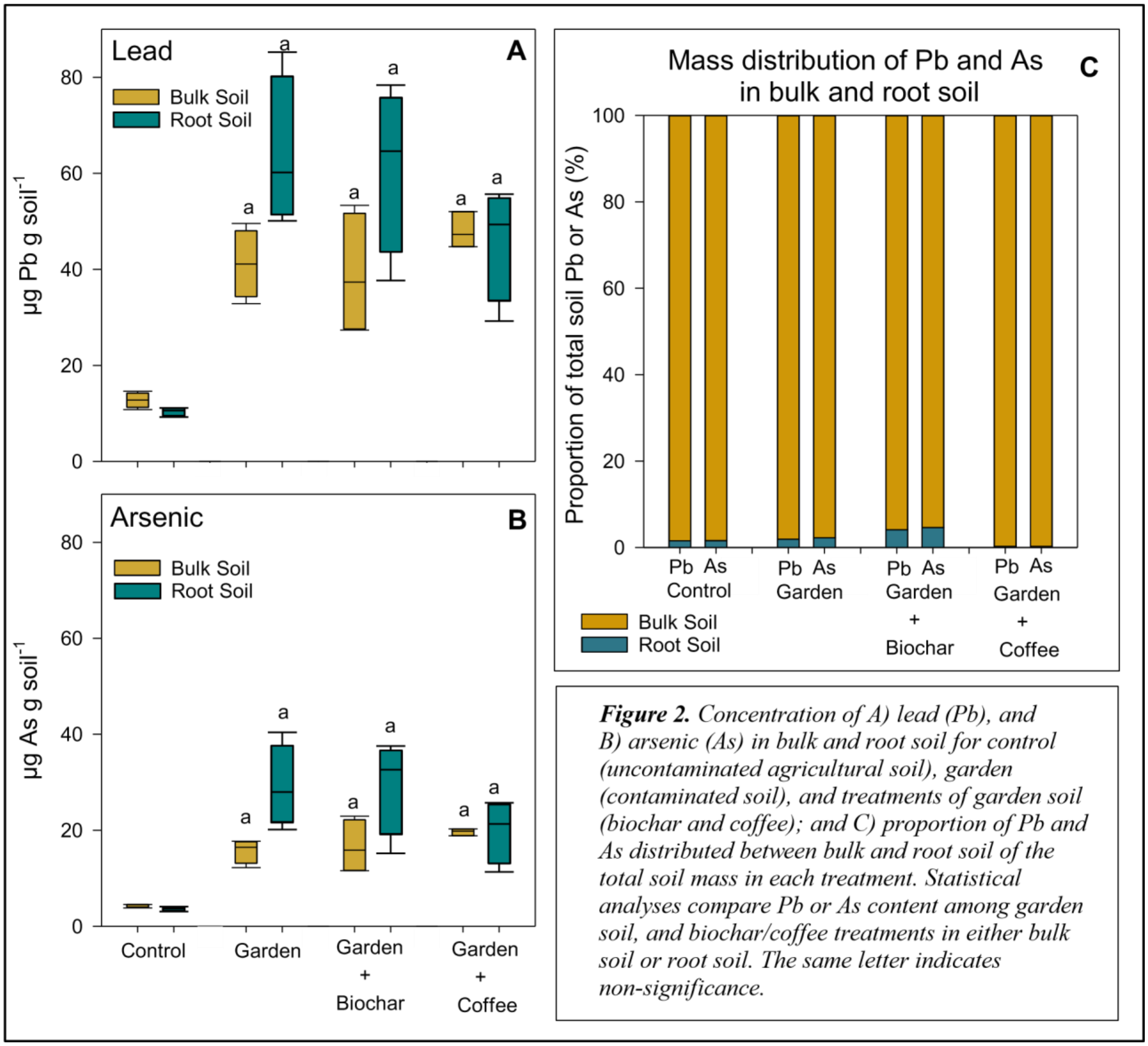

Figure 2. Concentration of A) lead (Pb), and B) arsenic (As) in bulk and root soil for control (uncontaminated agricultural soil), garden (contaminated soil), and treatments of garden soil (biochar and coffee); and C) proportion of Pb and As distributed between bulk and root soil of the total soil mass in each treatment. Statistical analyses compare Pb or As content among garden soil, and biochar/coffee treatments in either bulk soil or root soil. The same letter indicates non-significance.

Plants

Figure 3 looks at the distribution of As and Pb concentrations in the leaf, stem, and roots of the plant. The garden treatment plants had the largest overall size, based on visual appearance (Figure 4). The garden treatment also had the largest leaves (1.73-1.14 g) as compared to garden + biochar (0.51-1.17 g), and garden + coffee (0.05-0.83 g). The garden + coffee plants were significantly smaller than all other plants. (Figure 4) The garden treatment (0.29-0.58 g) also had more root mass than garden + biochar (0.13- 0.24 g), and garden + coffee (0.03-0.05 g). This pattern continues to the stem as well. Overall, the stem was the part of the plant with the least mass. The garden (0.3-0.4 g) had the most followed by garden + coffee (0.09-0.36g), then garden + biochar (0.008-0.014g). The control had the most mass in the leaves (0.79-0.9g), then roots (0.17-0.2g), and finally the stem (0.06-0.12g).

Lead and Plants

In general, the stem had the lowest accumulation of Pb and As. (Fig. 3A) The highest accumulation of Pb was in the roots, and leaves had medium Pb accumulation. (Fig. 3A) Statistics were not run on roots or stem due to their small masses. They were combined in order to get enough to perform the digestion. The garden treatment had the highest concentration of Pb in its roots (10.19 μg/g) followed by leaves (0.97-1.36 μg/g), and then stem (0.50 μg/g). Garden + biochar had the highest Pb concentration in its roots (3.42 μg/g), leaves (0.29-2.35 μg/g), and then stem (0.02 μg/g). (Fig 3A) The garden + coffee treatment had two Pb concentrations, the higher concentration of Pb was from initial plant leaves while the lower Pb concentration was from transplanted leaves. (Fig. 3A) Garden + coffee had the highest Pb concentration in its roots (8.22 μg/g), leaves (1.02-4.93 μg/g), and then stem (0.10 μg/g). The garden + coffee treatment had a significantly higher concentration of Pb in the leaves than the garden or garden + biochar treatment. (Fig. 3A)

Arsenic and Plants

Similar to Pb, the lowest accumulation of As was in the stem. The highest accumulation was in the roots, with a medium accumulation in the leaves. As stated above, there it was not possible to run statistics for the roots or stems. However, the garden + coffee had significantly higher accumulation of As in the leaves than the garden or garden + biochar treatment. (Fig. 3B) The garden treatment had the highest As concentration in the roots (7.0 µg/g), then leaves (0.97-1.36 µg/g), then stem (0.12-0.22 µg/g). This pattern followed for the garden + biochar treatment accumulations of As, roots (3.42 µg/g), then leaves (0.29-2.35 µg/g), then stem (0.02 µg/g). (Fig. 3B) The garden + coffee treatment had two As concentrations, the higher As concentration was from initial plant leaves while the lower As concentration was from transplanted leaves. (Fig. 3B) The garden + coffee treatment accumulations of As, roots (8.22 µg/g), then leaves (1.02-4.93 µg/g), then stem (0.10 µg/g). (Fig. 3B)

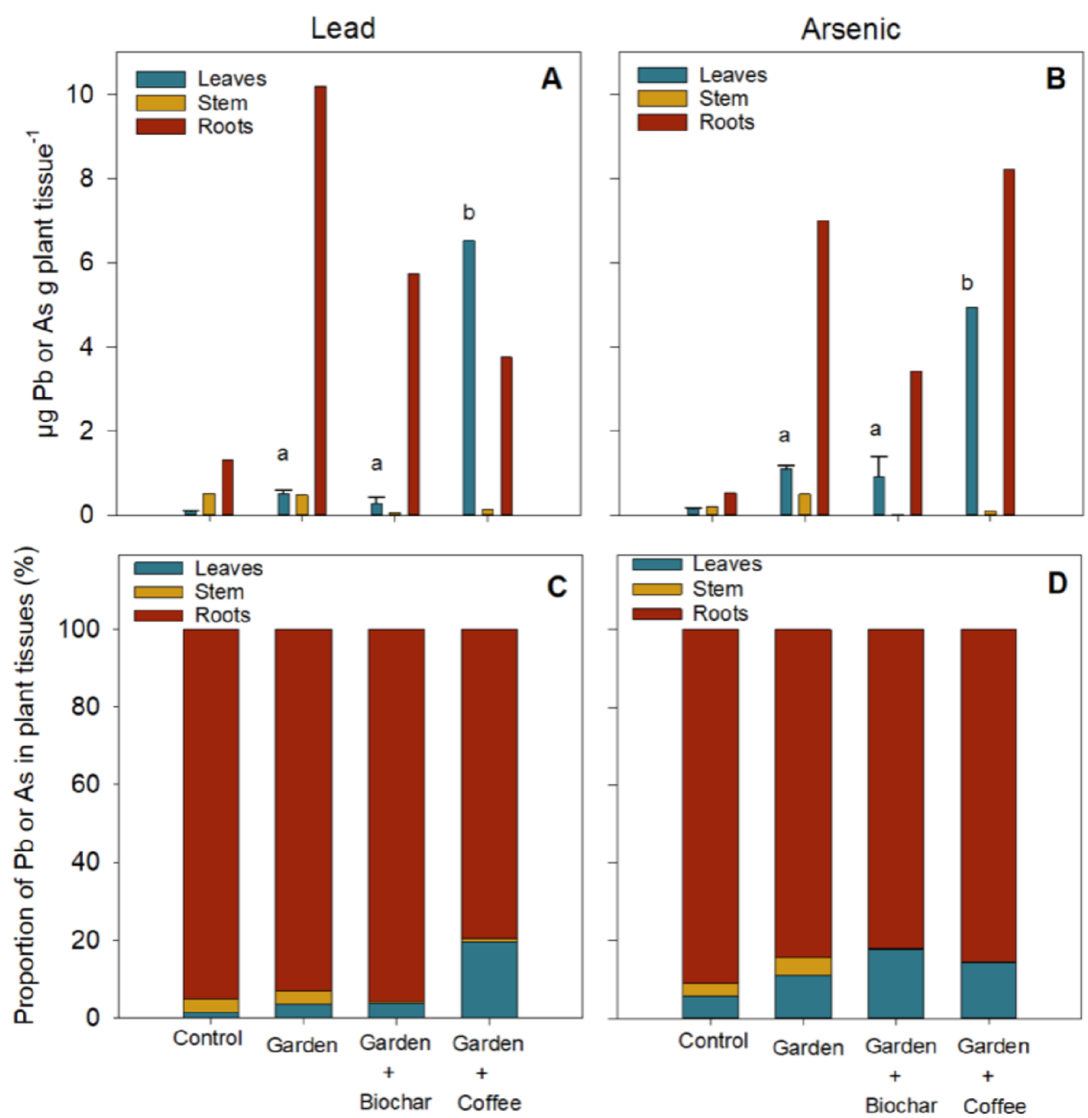

Figure 3. *Concentration of A) lead (Pb), and B) arsenic (As) in plants grown in control (uncontaminated agricultural soil), garden (contaminated soil), and treatments of garden soil (biochar and coffee); and proportion of C) Pb and D) As mass distributed between the plant leaf, stem, and root portions of the whole plant mass in each treatment. Statistical analyses compare Pb or As content among garden soil, and biochar/coffee treatments in either leaves, stem, or roots. The same letter indicates non-significance.*

Plant health and images

As can be seen in Figure 4, the different treatments had plants that resulted in drastically different sizes. The garden + coffee had significantly smaller plants, even after transplant than all other treatments. The garden + biochar had the largest plants. The garden and garden + biochar had the darkest green color. The garden + coffee plants had mostly brown or light-yellow leaves. The control had brown leaves and their leaves tended to be lighter green. Spots started to appear on the plants about halfway through their growing season. These spots started to appear when small black bugs made an appearance. The spots were mostly light brown but varied in color to white and black as well. After examining other papers that saw foliar necrosis due to atmospheric transfer of Pb (Uzu et al., 2010). The spots noted were light brown and mainly along the central nerve (Uzu et al., 2010).

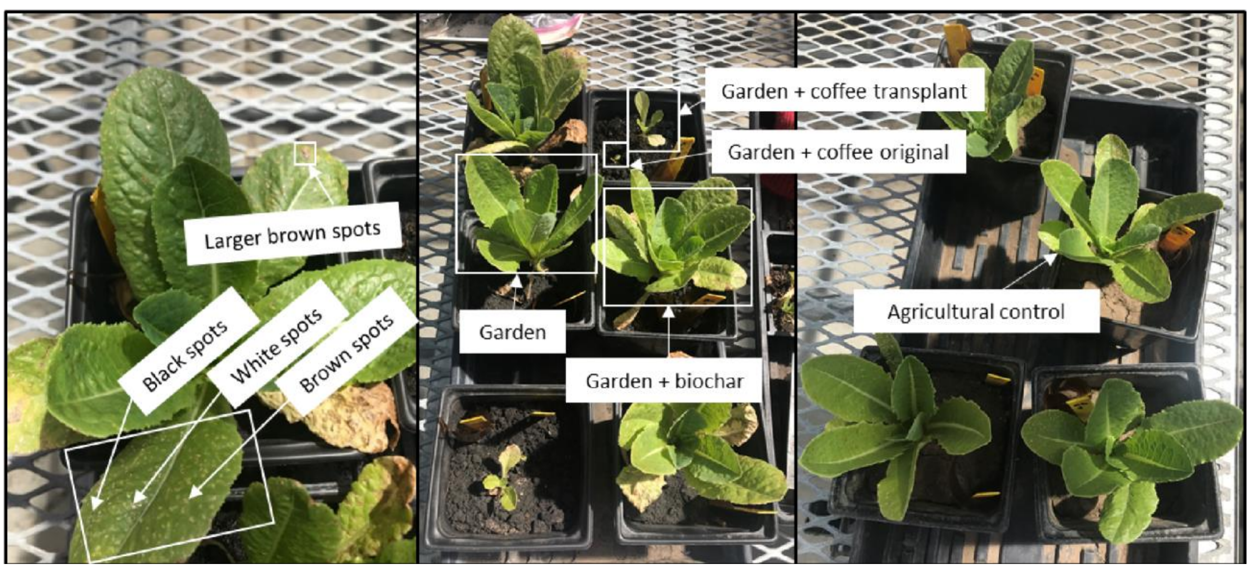

Figure 4. *Leaf spots on a garden + biochar plant on June 4, 2019, 8 weeks and 3 days after planting of the plants one day before harvest, June 10, 2019*

Whole system (soil and plant) comparison

Table 3 and 4 display the total percent of As and Pb distributed throughout the total system. The total system includes both the entire plant, bulk soil, and root soil. More specifically, there was a significant difference between the total percent of Pb in the root soil and the total percent of Pb found in the whole plant.

Table 3. *Distribution of lead in plant part and soil as a percentage of the total input mass.*

	Control	Garden	Garden + Biochar	Garden + Coffee
Bulk soil (%)	98.375	97.978	95.817	99.731
Root soil (%)	1.582	1.960	4.159	0.267
Total plant (%)	0.043	0.062	0.024	0.002
Leaves (%)	0.001	0.002	0.001	<0.001
Stem (%)	0.002	0.002	<0.001	<0.001
Roots (%)	0.040	0.058	0.023	0.002

Table 4. *Distribution of arsenic in plant part and soil as a percentage of the total input mass.*

	Control	Garden	Garden + Biochar	Garden + Coffee
Bulk soil (%)	98.316	97.587	95.307	99.7221
Root soil (%)	1.631	2.290	4.654	0.269
Total plant (%)	0.052	0.123	0.038	0.010
Leaves (%)	0.007	0.013	0.007	0.001
Stem (%)	<0.001	0.006	<0.001	<0.001
Roots (%)	0.032	0.104	0.032	0.009

Scanning electron microscope – energy dispersive x-ray (SEM-EDX)

Elemental mapping was performed using SEM-EDX on the leaf stem, and root of a lettuce plant gown in garden soil without treatment (Fig. 5A, B, C). Pb was observed to accumulate in some of the crevices of the root (Fig. 5C), however, all images displayed an absence of a strong pattern of distribution of both As and Pb. We did not observe necrosis or concentrations in the SEM-EDX images. Schreck et al. (2012) via SEM-EDX imaging found Pb alone and in conjunction with Fe^{3+}, Ca^{2+}, and K^{+}. The particles were all found along ridges in the leaf or near the stomata. These particulates were all found near the necrotic zones on plant surfaces. Nothing like this was found. (Fig. 5)

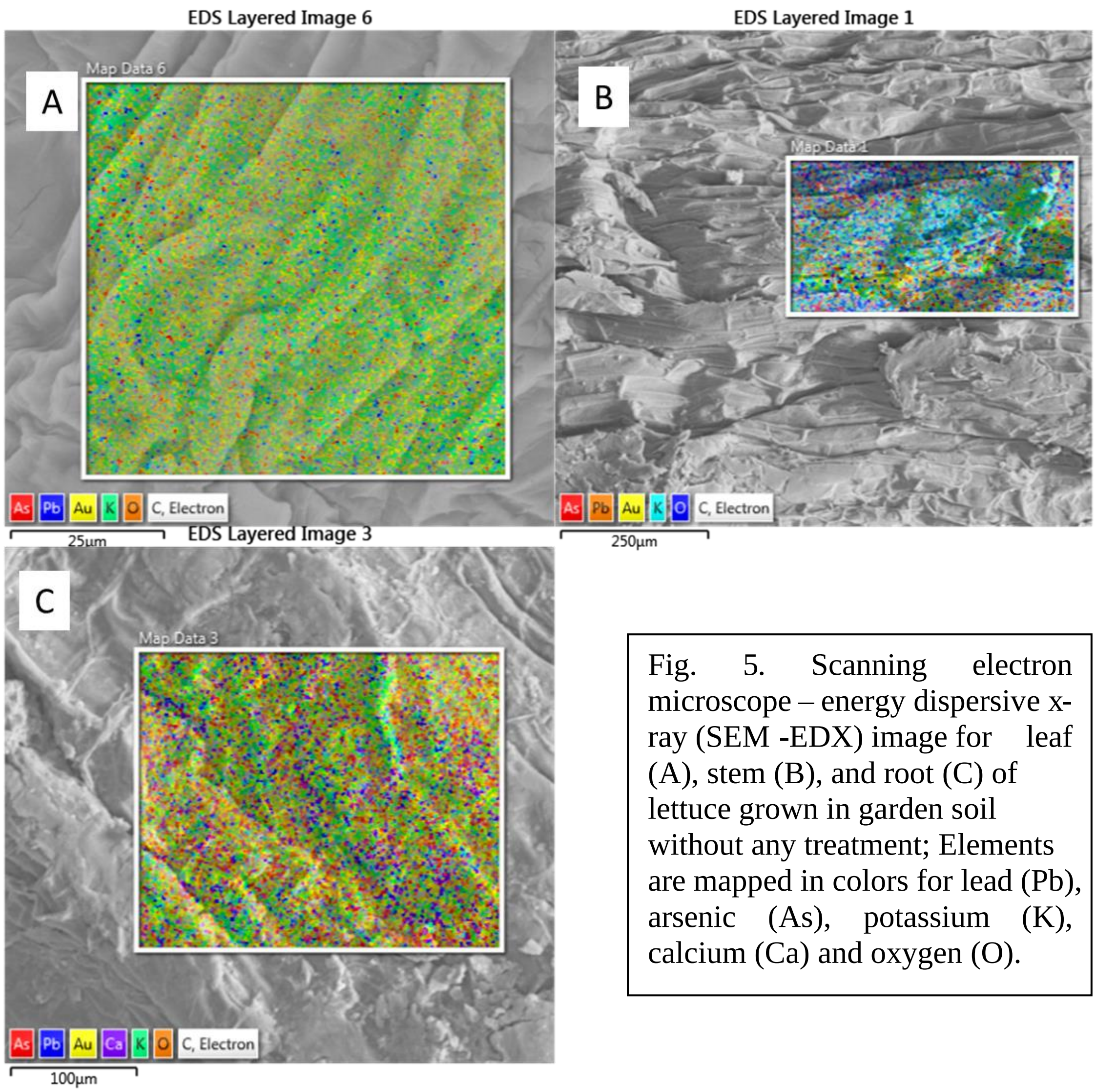

Fig. 5. Scanning electron microscope – energy dispersive x-ray (SEM -EDX) image for leaf (A), stem (B), and root (C) of lettuce grown in garden soil without any treatment; Elements are mapped in colors for lead (Pb), arsenic (As), potassium (K), calcium (Ca) and oxygen (O).

DISCUSSION

Coffee Grounds

I hypothesized that spent coffee grounds amendment to the contaminated garden soil would result in As and Pb^{2+} immobilization because of the above explained effects of the contained P^{-3}, Fe^{3+}, and Ca^{2+} ions. Furthermore, the chelators could attract Pb^{2+} ions, and they are a source of organic acids including oxalic acid which can facilitate Pb complexation.

The lowest proportion of both Pb and As in the root soil was found in the garden + coffee treatments. Less than 1% of Pb and As made it into the root soil. However, compared to all other treatments, the garden + coffee leaves were found to have significantly higher concentrations of As and Pb (Fig. 4A, Fig. 4C). The concentration of Pb was higher in the leaves than the roots. However, based on mass, the majority of both As and Pb was still in the roots. The As root concentration, in addition to the leaf concentration, was higher than all other treatments. Two things might be happening here, As and Pb^{2+} either not entering the root soil at the same rate as other treatments or once it is entering the root soil it is then being up taken into the plant. Based on the high concentrations of Pb and As in the roots and leaves, additional research needs to clarify Pb^{2+} and As pathways once it enters the root soil.

Highest concentrations of Pb^{2+} were found in conditions under pH 7 and highly alkaline conditions around pH 11 (Król, 2020). Due to the coffee grounds having a pH of 5.08, this is below a pH of 7. This could explain the significantly higher concentrations of Pb found in the leaves. The concentrations of Pb^{2+} in the root soil and bulk soil was lower in the garden + coffee treatment than the other two garden treatments. Pb^{2+} can be up taken by the plant either by calcium ion channels or apoplast pathways (Porrut et al., 2011). While the coffee grounds did input 347 mg/kg Ca (Table 2), Pb was still getting into the roots and leaves (Fig. 3). When there are high concentrations of Pb in the soil, it can destroy the Casparian strip in a plant (Porrut et al., 2011). With this destroyed it would allow for easier transportation of Pb to the leaves. The pH could have been a big factor too. Harter (1983), found that Pb retention does not start until above pH 5. The pH of the garden + coffee soil was 5.08 before planting, resulting in potentially less retention. Citric acid was found to be the most effective organic acid in mobilization of Pb^{2+}

in soil (Kim et al., 2013). Citric acid is found in coffee grounds (Borém et al., 2015). Potentially, the addition of the coffee grounds could have added in this mobilization. The coffee grounds were also significantly higher in K^+ than biochar or the soils. There was also an influx of Pb found in the leaves of the garden + coffee treatment. A study by Elouear et al., (2016) found an increase in Pb in the leaves of an alfalfa plant with applications of KCl fertilizer. Potentially, the high K^+ could be increasing this translocation of the Pb to aerial plant parts (Elouear et al., 2016).

The maximum release of As is found in acidic soils, especially under pH 3.0. This could be from the dissolution of soil iron oxides (Gersztyn et al.,2013). However, it is highly unlikely for a soil to have that low of a pH in nature, this pH level was manufactured by the addition of HCL and NaOH. (Gersztyn et al., 2013). While the coffee grounds may have been high in iron, the acidic conditions may have forced them to be leached and not available to bind with the As (Gerszytn et al. 2013, The University of Maine). Additionally, Marin et al., (1993) found that the majority of the As remained in rice roots, similar to our results. The roots are capable of expelling As which could result in the higher concentration. As can make its way to the leaves due to its resemblance to phosphate (PO_4^{3-}). It is then transported via the xylem into the leaves (Meadows, 2014). This could be a reason we saw higher concentration in the leaves and roots than the stems. While the coffee grounds themselves were high in P^{-3} (Table 2), P^{-3} is less bio-available at pH below 5.5 (USDA). This is due to high fixation with aluminum and iron, which could be another reason that the As was higher in availability because the P^{-3} was immobilized with the Fe^{3+} instead (USDA). Additionally, the coffee grounds increased moisture retention which could have facilitated more bacterial growth and certain types of bacteria can facilitate transformation of As into bio-available forms (Abbas et al., 2018).

It should be kept in mind that we needed to transplant lettuce plants because of delayed germination in the coffee treatments. Some of the issues with the decreased size of the lettuce plants could have come from the low pH, increased moisture retention, or potentially from caffeine toxicity. Caffeine toxicity to plants was also observed by Hardgrove & Livesley, (2016), who found that spent coffee grounds, when added directly to the soil, inhibited seed germination and decreased plant growth. Applications with more than 10% spent coffee grounds resulted in greater growth inhibition (Hardgrove & Livesley, 2016). Our application averaged around 10%. Gomes et al., (2014) confirmed this, who also found that the inhibition of lettuce seed germination could have come from caffeine toxicity. If I were to use coffee grounds as an amendment again, I would decrease the amount applied and compost them before addition. Gomes et al. (2014) found that when the coffee grounds were composted, there was no growth inhibition below a 10% application rate.

Biochar

Biochar was hypothesized to immobilize As or Pb because of an increase in soil pH, decrease in zeta potential, increase in CEC to attract positively charged Pb^{2+}, the available compounds to create Pb and As complexes, the available surface area for Pb and As adsorption.

There was facilitated movement in the garden + biochar treatment from bulk to root soil at a similar rate as in the garden soil. In the garden + biochar treatment, there was little movement from root soil to roots, stem, and leaves.

The pH of the biochar amendment was 7.7. This could have decreased the solubility of As. In the future I would make sure to have a biochar that either had a pH > 7 or pH < 3. To decrease the solubility of As as much as possible. The biochar As decreased percent, found in the roots could be due to the addition of P^{-3} through the biochar amendment. As is an analog for P^{-3}. This means that As can use the P^{-3} channels within lettuce for uptake (Zhao et al., 2009). There is conflict within research if increased P increases or decreases As adsorption. The biochar had 13 mg/kg P^{-3}. P^{-3} volatizes at 700˚C (Zhang et al., 2016). The biochar was prolysized at 760˚C. In the future, I would try biochar pyrolysized below 700˚C, to increase the overall available P^{-3}. There is contradicting research on if P would decrease or increase the adsorption of As. There needs to be further studies in this area and a lower temperature prolysized biochar might provide that opportunity (Meharg et al., 2002, Moreno-Jiménez et al., 2012). Additionally, the additions of Fe^{3+} could increase binding sites for As (Zhang et al., 2005).

The biochar added a organic material to the soil. Pb^{2+} binds to organic material easily and therefore removes it for plant uptake (Porrut et al., 2011). Pb^{2+} decreases at higher pH and is retained at pH of 7.7 (Harter, 1983). The Pb was not entering the roots, this is in part due to the increased surface area and potential binding spots for Pb^{2+}. A 5% addition of biochar was found to decrease Pb^{2+} availability (Puga et al., 2015). Our amendment was 14%. The most stable forms of Pb are lead sulfides, lead phosphates, lead carbonates, and lead hydroxides (Wuana & Okieimen, 2011). The biochar amendment added 13 mg/kg of P^{3-} and 3.1 mg/kg sulfate (SO_4-S) (Table 2). This may have made some difference. However, the biochar was 48.61% carbon. Adding significant carbon to the soil (Table 2). This addition of carbon with the high surface area and higher pH could have effectively worked to retain Pb^{2+} in the soil.

The Food and Drug Administration (FDA) suggests 3 µg per day as a maximum daily intake level of Pb for children, and 12.5 µg per day for adults (FDA, 2018). In comparison to the levels found in the lettuce leaves, children and adults would not be able to consume large amounts before reaching the FDA maximum suggested threshold. Based on a 2,000 calorie diet the FDA has one serving of leaf lettuce at 85 g, about 1.5 cups, shredded. None of our treatments produced enough plants to reach that amount of lettuce. The garden soil treated leaves was 49 g with a combined total of 0.21 µg Pb/ 85 g. If there was 85 g from the garden soil treated leaves, there would be an estimated 1.22 µg Pb/ 85g. For the biochar treatment there would be 0.41 µg Pb/ 85g in one serving of lettuce. For the coffee treatment there would be 7.42 µg Pb/ 85g. Far exceeding the daily maximum for children.

There is not a specific recommendation for daily intake of As. Levels over 10 mg/kg daily can cause arsenicism. Arsenicism also known as blackfoot disease can lead to loss of your extremities (United States Institute of Medicine Panel on Micronutrients, 2001). According to, (United States Institute of Medicine Panel on Micronutrients (2001) “the median intake of As by men and by women was approximately 2.0 to 2.9 µg/day and 1.7 to 2.1 µg/day, respectively.” In one serving (85 g) of lettuce from the control there would be 0.35 µg As/85 g. The garden treatment would have 2.78 µg As/ 85 g. The biochar treatment would have about 1.57 µg As/85 g serving of lettuce. The coffee treatment would have 10.76 µg As/85 g. Eating a serving of lettuce grown in the garden soil or garden + biochar treatment would not result in a level that could cause arsencism. However, eating a serving of lettuce grown in the garden + coffee treatment could result in levels that lead to arsencism.

Experimental considerations

For future experiments, I would recommend ensuring that there were no pests on nearby plants before starting. Another recommendation would be to grow the transplants in the same soil as the controls. For the control soil, I would use soil that is conducive to lettuce plants such as a greenhouse soil, rather than a soil used for corn. The use of transplants worked well because all replicates had plants in them, even ones that did not germinate within the first few weeks. The water schedule and watering by weight was easy to record and ensured that all plants, had the adequate water, at all times. The method used for digestion worked well even though some of the plants were smaller. The charcoal pieces found in the garden soil most likely came from the neighboring trains that passed right next to the lot. The control bulk and root soil showed on average levels of 23.17 mg/kg of Pb^{2+}. The control root and bulk soil made up over 99% of the Pb total of the plant and soil system. An 83 sample United States Geological Survey of Nebraska's soils found an average Pb level of 18.2 mg/kg (USGS, 2010). A control soil that is closer to the state average might be better suitable for comparative analyses because of better representation of the levels occurring across the state. Also, more replications to ensure that there is less variability within the soil. To ensure that the treatments are as homogenous as possible. The EPA standard for 99% of uncontaminated soil is less than 13.7 mg/kg As and the average level for uncontaminated soil is 7 mg/kg As (EPA, 2005.) Similar to Pb, the control root and bulk soil made up over 99% of the As total for the plant, soil system, with an average level of 7.97 mg/kg As, which is representative.

CONCLUSIONS

Community gardens are a good option to help sustain neighborhoods where food deserts exist. However, these areas can be in industrial areas or near railways or large throughways that result in increased pollution. This pollution can result in deposition of heavy metals onto soil surfaces. This heavy metal build-up can eliminate potential garden areas, due to the heavy metals entering the food chain via planting in contaminated soil. This study aimed to find an affordable solution to ensure that Pb and As, two heavy metals commonly found in soil, were immobilized and did not enter into food sources. Soil amendments can easily be applied by any individual. While fresh spent coffee grounds did not prove to be effective in immobilizing As and Pb^{2+} and resulted in plants too small to be a food source. I am hopeful that they could still be a potential soil amendment if charred or composted. This is in part due to the effectiveness of the biochar in immobilization. If there were soils low in potassium, magnesium, calcium, or were too basic, an addition of coffee grounds with another amendment could be helpful for remediation. Coffee grounds could be particularly useful to encourage mobilization of Pb and As with the use of hyperaccumulator plants. In a community garden setting, a coffee ground soil amendment with planting a bio accumulator plant could remove Pb and As. Then a biochar amendment could be added to the soil to immobilize any remaining Pb and As, resulting in safe soil levels for gardens. This experiment would need to be taken into a real-life community garden plot and see the potential effects that a soil amendment could have, due to the larger and more complex scale of a field experiment.

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